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Oxidation of Hydroxylamine Derivatives. IV.[1] Anodic Oxidation of Cycloserine and Its Acetyl Derivatives in Aqueous Buffer Solution

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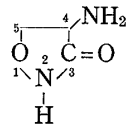
Anodic oxidation of cycloserine and its acetylderivatives was studied at a glassy-carbon electrode in alkaline aqueous buffer solution. On electrolysis, cycloserine consumed one Faraday per mol and yielded about 50% serine, 10% ammonia and a small amount of resinous products. A possible reaction scheme for the anodic oxidation of cycloserine is proposed.

Keywords——cycloserine; N-acetyl and N,2-acetylcycloserine; cyclic voltammetry; anodic oxidation; glassy-carbon electrode; N-N coupling; serine; deamination

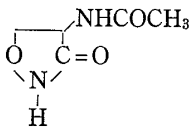
Cycloserine (I), a broad spectrum antibiotic, has a cyclic O-alkyl hydroxamic acid structure. This compound is unstable in neutral or acidic solution, decomposing to hydroxylamine and serine upon acid hydrolysis,[3] whereas it is stable in aqueous alkaline solution and shows an anodic oxidation wave.

In this paper we describe the anodic oxidation of cycloserine and its derivatives studied by cyclic voltammetry, as well as its controlled potential electrolysis in aqueous alkaline buffer.

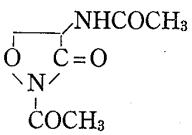
Compounds used in this study are as follows.



cycloserine (I)



N-acetylcycloserine (II)



N,2-diacetylcycloserine (III)

Results and Discussion

Cyclic Voltammetry

Cycloserine showed a well-defined anodic peak at about 0.65 V in aqueous buffer solution of pH 10 at a glassy-carbon electrode, though serine and simple α-amino acids do not develop an anodic wave under similar conditions. The height of the wave corresponds to one-electron oxidation as defined by comparing its peak current with that of ephedrine ($n=2$).

The peak potential (E_p) *vs.* pH profile is shown in Fig. 1.

The profile shows breaks at about pH 5 and 8 which are close to pK_1 (4.40) and pK_2 (7.40)[3c] of cycloserine, respectively. In the pH range above 10 the E_p is almost pH-independent. Thus, this profile suggests the presence of the following forms in equilibrium.[3c]

1) Part III: S. Ozaki and M. Masui, *Chem. Pharm. Bull.* (Tokyo), **27**, 357 (1979).
2) Location: *133-1, Yamada-kami, Suita-shi, Osaka.*
3) *a*) F.A. Kuehl, F.J. Wolf, N.R. Trenner, R.L. Peck, E. Howe, B.D. Hunnewell, G. Dowing, E. Newstead, R.P. Buhs, I. Putter, R. Ormond, J.E. Lyons, L. Chaiet and K. Folkers, *J. Am. Chem. Soc.*, **77**, 2344 (1955); *b*) G.M. Conzerman, Jr., *Antibiot. Chemother.*, **5**, 444 (1955); *c*) T. Tsukamoto and S. Ozeki, *Yakuzaigaku*, **27**, 336 (1967).

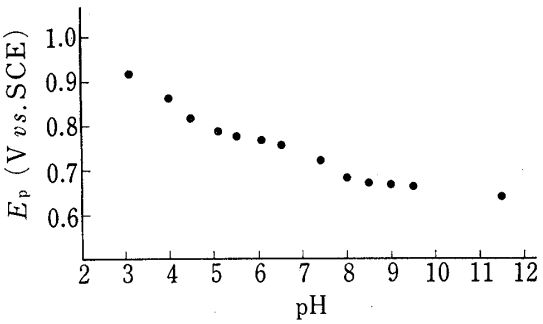
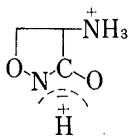


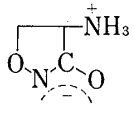
Fig. 1. Cyclic Voltammetric E_p-pH Behavior of Cycloserine at a Glassy-Carbon Electrode in Aqueous Buffers

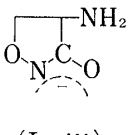
Table I. Cyclic Voltammetric Data for Cycloserine and Its Acetyl Derivatives at a Glassy-Carbon Electrode in Aqueous Buffer, pH 10

Compds.	E_p (V *vs.* SCE)
I	0.64
II	0.65
III	2.00[a] 2.40[a]

a) In acetonitrile containing 0.1M $NaClO_4$.


(I−i)


(I−ii)


(I−iii)

Since O-methylhydroxamic acid under basic conditions shows the first anodic wave at a potential close to that of cycloseine,[4] and aliphatic primary amines show an ill-defined anodic peak at 1.45—1.55 V, the peak potential of 0.65 V suggests that the first electron transfer occurs from the region with negative charge in (I-iii) rather from the free amino group.

As shown in Table I, N-acetylcycloserine (II) showed an anodic peak at a potential nearly equal to that of cycloserine, while N,2-diacetylcycloserine (III) did not show any anodic peak in the potential range covered by the present system.

Controlled Potential Electrolysis

As shown in Table II, on controlled potential electrolyses of (I) and (II) at potentials slightly more anodic than their peak potentials, the n value was found to be about one; DL-serine (yield about 50%) and ammonia (yield about 12%), together with a small amount of unidentified resinous products, were obtained.

Table II. Controlled Potential Electrolysis of Cycloserine (I) and N-Acetylcycloserine (II)

Compds.	pH	$E_{app.}$[a]	n[b]	Product	Yield (%)[c]
I	10.3	0.80	1.0	DL-Serine	48
				Ammonia	12
II	10.2	0.75	1.0	N-Acetylserine	41

a) Applied potential in volts *vs.* SCE.
b) Coulombs passed per mol of the substrate.
c) Mol per cent on the basis of the starting material.

The yield of ammonia increased to 18% on leaving the solution to stand at room temperature overnight after electrolysis. Under the same conditions, DL-serine also produces ammonia, so a part of the ammonia may be derived from the decomposition of DL-serine produced. Nearly the same results were obtained with a platinum gauze electrode. In the pH range from 8.27 to 11.2 and at applied potentials from 0.65 to 1.0 V the same products were obtained in similar yields.

4) S. Ozaki and M. Masui, *Chem. Pharm. Bull.* (Tokyo), **25**, 1179 (1977).

No other product containing nitrogen such as nitrous oxide or nitrate anions, was detected.

N-Acetylcycloserine (II) also showed an n value of about one and gave N-acetylserine in 41% yield.

These results suggest that cycloserine undergoes one-electron oxidation; about half of the generated intermediate forms serine, while the other half gives unidentified products. A reaction process which includes the reaction of two-electron oxidized cycloserine with unoxidized cycloserine seems unlikely, because the cyclic voltammograms of cycloserine showed a peak current corresponding to one-electron transfer.

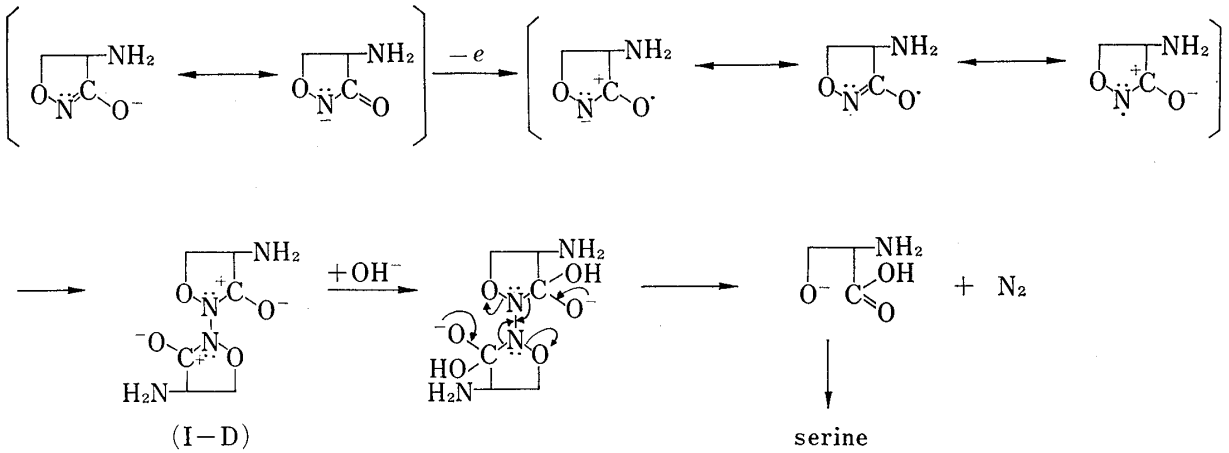
Table III shows that the yield of DL-serine throughout the electrolysis is about half of the amount of cycloserine (I) oxidized.

Table III. Serine Production during the Controlled Potential Electrolysis

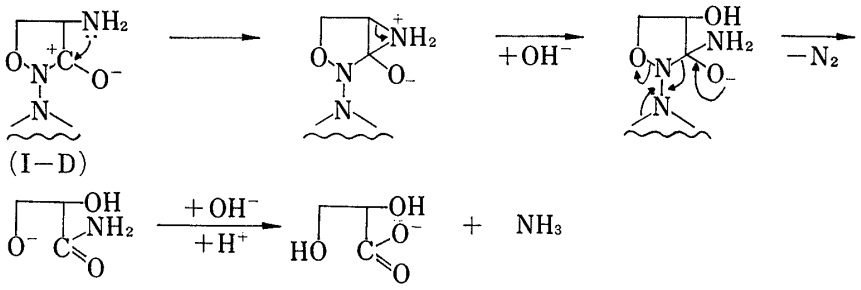
(*A*) Cycloserine oxidized (%)	(*B*) Serine produced (%)	(*B*)/(*A*)
13.4	5.77	0.43
34.7	22.1	0.64
48.9	29.6	0.61
78.7	39.2	0.50
95.7	44.0	0.46
96.2	48.0	0.50

Hydroxylamine, reported to be the hydrolysis product of cycloserine,[3a] shows two anodic waves at 0.60 V and 0.90 V at pH 10, and on controlled potential electrolysis it gives n values of 2.2 and 4.3 at 0.60 V and 0.80 V, yielding about 6.5% and 76% nitrate ions, respectively. In the course of the anodic oxidation of cycloserine at 0.80 V, the generation of hydroxylamine is, therefore, unlikely, since the coulometric n value observed on the oxidation of cycloserine is about one and no nitrate was detected.

The nitrogen atom in the isoxazolidinone ring of cycloserine may yield nitrogen gas through N-N coupling as observed in the anodic oxidation of O-methylhydoxamic acid.[4] On the basis of the present results, the following reaction scheme is proposed for the anodic oxidation of cycloserine.



Intramolecular nucleophilic attack of amino nitrogen instead of the oxygen of OH^- or water on the positively charged carbon of (I-D) should be possible, and the hydrolysis of the resulting aziridine ring of the carboximide would result in liberation of ammonia as shown in the following scheme.



The possibility of anodic oxidation of the amino group in cycloserine to form ammonia, cannot be excluded, since the anodic waves of primary amines (E_p; *ca.* 1.50 V) are rather ill-defined and drawn out over an appreciable potential range.

As glyceric acid was not obtained in the present experiments, it is not yet clear which mechanism is responsible for the yield of ammonia.

Experimental

Materials——D-Cycloserine (I) was obtained from Sumitomo Chemical Industry Co., Ltd. Acetylcycloserine (II) was prepared according to the method of Kuehl,[5] mp 178° (lit. 175—177°). N,2-Diacetyl-cycloserine (III) was prepared according to the method of Milne,[6] mp 119—121° (lit. 121—122°). Reagent grade D- and DL-serine were used without further purification.

Cyclic Voltammetry——Cyclic voltammograms were obtained with a three-electrode system. The electrode system consisted of a glassy-carbon indicator electrode, a glassy-carbon counter electrode, and a saturated calomel electrode (SCE). Measurements were carried out at 25±0.05° with a substrate concentration of *ca.* 5 mM and a sweep rate of 0.05 V sec^{-1}. After each run, the working electrode was rinsed with water and subjected to electrical pretreatment, *i.e.*, two voltage sweeps from *ca.* −0.1 V to *ca.* 1.5 V in a buffer solution of pH *ca.* 7.45. The electrode surface had to be repolished after runs to obtain reproducible results. Britton-Robinson buffer solution was used in cyclic voltammetry to obtain the E_p-pH profile.

Controlled Potential Electrolysis and Products Analysis

Apparatus and Procedures——Electrolyses were performed with a Hokuto Denko HA 101 potentiostat; the current was recorded on Toa Dempa EPR-2TB recorder. An H-type electrolysis cell was used; the anode compartment was separated with an agar plug and a fritted glass disk. The anolytes (*ca.* 1×10^{-2} M substrates) were electrolyzed using a glassy-carbon plate or platinum gauze electrode. 0.1 M carbonate buffer solution was used in controlled potential electrolysis.

Isolation and Determination of Serine——The electrolyzed solution was passed through a 2.5×40 cm column of H-form Amberlite CG-120 resine (100—200 mesh) and eluted with a buffer solution of pH 3.4. The buffer components of the electrolyzed solution and the unoxidized cycloserine were removed by this procedure. A part of the eluted solution was examined by paper chromatography using *n*-butanol–acetic acid–water (12:3:5), with ninhydrin test solution (0.2% (w/v) in 5% acetic acid-ethanol). The *Rf* value of *ca.* 0.2 was the same as that of authentic D-serine. The remainder of the eluted solution was concentrated and reacted with 1-fluoro-2,4-dinitrobenzene according to the method of Levy.[7] The resulting yellow crystals showed NMR and IR (KBr disk) spectra identical with those of the 1-fluoro-2,4-dinitrobenzene derivatives of D-serine and DL-serine, respectively.[9] Quantitative analysis of serine and N-acetylserine was performed according to the method of Akabori.[8] Cycloserine and N-acetylcycloserine did not interfere with the measurements.

Determination of Other Products——Ammonia was determined by means of the phenol-hypochlorite reaction.[10,11] Cycloserine was determined from the absorption in the ultraviolet region, $\lambda_{max}^{H_2O(pH=10)}$ nm(ε): 226 (3430).

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